TUBERCULIN-TREATED GUINEA-PIGS IN THE RECOGNITION OF TUBERCULOSIS.

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The experiments upon which this paper is based were undertaken in order to determine to what extent the tuberculin reaction in guinea-pigs might be of value in the diagnosis of human tuberculosis. The problem which presented itself was to reduce the length of time required for the determination of tuberculosis in guinea-pigs that had been inoculated with material containing tubercle bacilli.

The method now most commonly used is to introduce the suspected material either into the peritoneal cavity or the subcutaneous tissue of the abdomen of a guinea-pig, and after four to eight weeks examine the animal for tuberculosis. Block[1] modified this procedure by introducing the material in the region of an inguinal gland and then, by squeezing the gland, so lower its resistance that he could recover bacilli in nine to eleven days. F. Klemperer,[2] by a similar method, except that he did not squeeze the lymph gland, found tubercle bacilli in stained sections of it in ten to fourteen days. He found, in using Block's method that the pigs often died of mixed infection. Nattan-Larrier found that if virulent tubercle bacilli were injected into the base of the mammary gland of a lactating guinea-pig the bacilli could be found in the milk in from five to ten days. An indirect test has been described by Mérieux,[3] who obtained the tuberculin reaction in tuberculous guinea-pigs after the injection of serum from a tuberculous patient.

Anderson,[4] while determining the presence of tubercle bacilli in milk, found if he injected 2 c.c. of crude tuberculin into guinea-pigs two months after they had been inoculated with tubercle bacilli, that death almost invariably followed in six to eighteen hours. Guinea-pigs with slight lesions in two or three instances did not die, but became sick. All of the animals died when the lesions had caseated. As much as 7 c.c. given to a healthy guinea-pig caused only temporary discomfort. Anderson believes this test of distinct service in differentiating tubercle bacilli from other acid-fast bacilli. The statement has been made by Wolff[5] that while 0.5 to 2 c.c. of tuberculin will be tolerated without discomfort by a healthy guinea-pig, an injection of 0.1 to 0.15 c.c. will cause the death of a tuberculous pig within twenty-four to forty-eight hours. Attempts to utilize the von Pirquet reaction in guinea-pigs have been nearly uniformly failures.

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[1] Berl. klin. Woch., 1907, No. 17, 511.

[2] Ibid., 1908, 171.

[3] Revue de méd. 1906, xxvi, 2.

[4] Hygienic Laboratory, Bulletin, No. 41, United States Public Health and Marine Hospital Service, January 1, 1908.

[5] Berl. klin. Woch., 1908, 297.

I endeavored first to determine how soon after being inoculated a pig would die from the tuberculin reaction. Injections of tuberculin in doses of 0.25 c.c. and 0.5 c.c. given to healthy guinea-pigs resulted in no appreciable effects. Three guinea-pigs were then inoculated under the skin of the abdomen with an emulsion of very small numbers of tubercle bacilli. At the end of eight, twelve, and fifteen days the animals were given in turn 0.25 c.c. of tuberculin. The one given tuberculin on the eighth day showed no effects at the end of twenty-four hours, and even gained slightly in weight. The one given tuberculin on the twelfth day was sick the next day, but recovered. The nodule at the site of inoculation was larger than it had been the day before, and the pig lost slightly in weight. The one given tuberculin on the fifteenth day was sick the two following days and in other respects behaved like the one preceding, losing slightly in weight and showing a larger harder nodule on the abdomen. All three pigs were autopsied on the seventeenth day. In each there was slight caseation at the site of inoculation and much serous exudate in the adjacent cellular tissue. In this exudate, as well in the caseous material, very large numbers of tubercle bacilli were found. In the pig that had received tuberculin on the eighth day there were miliary tubercles in the liver and spleen. In all the pigs the inguinal lymphatic glands were caseous and contained many tubercle bacilli.

Having failed to get satisfactory results by awaiting the death of the guinea-pigs, but judging that the very large numbers of bacilli found at autopsy were in some way due to the tuberculin, a second series of pigs was inoculated with 0.5 c.c. of an emulsion of tubercle bacilli made so dilute that only two or three bacilli could be found microscopically in an hour's search. These pigs were given 0.25 c.c. of tuberculin at various intervals and chloroformed and autopsied at the end of twenty-four hours. In this manner tubercle bacilli were recovered from the point of inoculation in the subcutaneous tissue as early as the sixth, seventh, eighth, and ninth days. Pigs autopsied on the fourth and fifth days were negative. In one killed on the third day three bacilli were found in a search of an hour and a half. A dose of 0.25 c.c. of tuberculin is such an overwhelmingly large one for a guinea-pig that it was found that size or age of the animal made no difference in the results.

Attention was then given to the possible clinical value of the foregoing suggestions. Pigs were inoculated with suspected material by injecting an emulsion of it under the skin of the left side of the abdomen, the other side being left for the subsequent subcutaneous injection of tuberculin. Each pig was chloroformed and autopsied twenty-four hours after the latter injection. The results were as follows:

1. Pus from empyema. Microscopically no tubercle bacilli; 2.5 c.c. of emulsion of this injected into pig. Tuberculin sixth day. Autopsy, seventh day. A few tubercle bacilli found at site of inoculation.

2. Fluid from tenosynovitis of knee of long standing. No tubercle bacilli microscopically. Tuberculin sixth day. A few tubercle bacilli found at site of inoculation on the seventh day.

3. Suspected tuberculosis of the knee of two years' duration. Not possible to demonstrate tubercle bacilli in fluid from the joint. First pig given tuberculin on sixth day. Autopsy on seventh day. Negative result. Second pig given tuberculin on fourteenth day. Death of animal in twenty hours from reaction. Very large numbers of tubercle bacilli at point of inoculation, which showed caseation and much serous exudate. There were also miliary tubercles in the liver and spleen.

4. Suspected tuberculosis of the ankle-joint. Scrapings emulsified and injected. No tubercle bacilli microscopically. Tuberculin on seventh day. A few tubercle bacilli found at autopsy on the eighth day.

5. Suspected tuberculosis of the kidney. Patient a woman, aged thirty-four years, considerably emaciated, slight irregular fever and a large tender displaced right kidney. The urine contained pus and blood and very large numbers of acid-fast bacilli which were decolorized by alcohol. Two pigs inoculated. Both given tuberculin on the fourteenth day. An abscess had formed under the skin. Pus from this showed no tubercle bacilli after the tuberculin. Pigs killed six weeks after inoculation showed no tuberculosis. Patient has recovered.

A second group showed negative results:

1. Scrapings from fistula into suspected tuberculous hip-joint. Pigs examined on seventh and sixteenth days.

2. Scrapings from fistula into suspected tuberculous hip-joint. Pig examined on seventeenth day.

3. Fluid from symptomatic hydrocele in case of suspected tuberculous testicle. Sections of the testis did not reveal tuberculosis. Pigs examined on the seventh, fifteenth, and thirty-first days.

4. Emulsion from caseous peribronchial gland, which contained tubercle bacilli microscopically, from a child dead of tuberculous meningitis. One pig examined on the eleventh day. The failure in this case suggested the possibility that the child had died of bovine tuberculosis and that tuberculin from human bacilli had no effect.

Of the 62 guinea-pigs used, none died of mixed infectious. One died from tuberculin reaction on the fourteenth day. One with advanced pulmonary tuberculosis of eight weeks' duration, in which there was caseation, survived 0.25 c.c. of tuberculin four days. Pigs No. 51 and 52 were inoculated with the same quantity of the same material at the same time. Both were autopsied on the seventeenth day, one having been given tuberculin the day before. In both tubercle bacilli were found at the point of inoculation, but by far the larger number were present in that pig to which tuberculin had been administered. It appears that overwhelming doses of tuberculin given to a guinea-pig the subject of tuberculosis breaks down its resistance to the tubercle bacilli and results in their much more rapid multiplication.

The method employed in carrying out this test has simplicity to commend it and the fact that positive results are conclusive. The technique consists of making an emulsion of the suspected material in normal salt solution, if it is not already fluid enough to be injected through a course syringe or sharp-pointed improvised pipette. Three guinea-pigs are inoculated by injecting a considerable quantity under the skin of the abdomen. At the end of six days one pig is given 0.25 c.c. of tuberculin hypodermically on the other side of the abdomen, and is chloroformed at the end of twenty-four hours, and autopsied. The fluids at the site of the original inoculation are smeared upon a slide and examined in the ordinary way for tubercle bacilli. If the first pig results negatively, another is examined in the same way by giving tuberculin at the end of two weeks, and making the autopsy at the end of twenty-four hours. If this is also negative, the third pig is given tuberculin at the end of four to six weeks, and the result of its examination is a check upon the first two.

The test offers a means, in a considerable proportion of cases, of making a diagnosis at the end of a week instead of awaiting until the lapse of four to eight weeks. It also presents a method of distinguishing tubercle bacilli from other acid-fast bacilli, especially when, as often happens, they are present in large numbers.